# Basic Matters Concerning Methods of Animal Experiments, etc.

National Cerebral and Cardiovascular Center
Research Promotion Support Department
Animal Experiment Management Office

Kyoko Shioya


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# Drafting an animal experiment protocol Approval/cost/implementation/results

# General flow

- Attend education and training
- Consideration of research direction
- cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

# General flow

- Attend education and training
- Consideration of research direction
- Cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

- Confirmation and compliance with relevant laws and regulations
- Confirmation of facility rules
- Confirmation of necessary procedures

# General flow

- Attend education and training
- Consideration of research direction
- Cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

- Literature search
- Purpose of the experiment Assignment of roles
- Rearing environment confirmation
- Confirmation of alternative methods

# General flow

- Attend education and training
- Consideration of research direction
- Cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

- Research funding
- Research period

# General flow

- Attend education and training
- Consideration of research direction
- cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

- Ethical considerations
- Experimental procedures
- Investigation of balance between degree of pain and outcome
- Investigation of humane endpoints
- Investigation of euthanasia method
- Examination of the number of animals used

# General flow

- Attend education and training
- Consideration of research direction
- cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

- Implementation period
- Confirmation of breeding facilities
- Breeding management
- Quarantine/microbial monitoring Waste treatment

# General flow

- Attend education and training
- Consideration of research direction
- Cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

- Dissection for data acquisition
- Sample collection
- Tissue analysis
- Analysis of samples such as blood

# General flow

- Attend education and training
- Consideration of research direction
- cost of implementation
- Formulation of animal experiment protocol
- Conducting animal experiments
- Analysis of acquired data
- Formulation of reports on animal experiment results
- Paper writing

Submit according to the facility's agreement, such as after the end of the experiment or after the end of the period.

# General flow

- Attend education and training
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- Formulation of reports on animal experiment results
- Paper writing

Efforts will be made to communicate the results obtained from animal experiments with the ultimate goal of providing feedback to human health.

# PREPARE

norecopa

## The PREPARE Guidelines Checklist

### Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith[a], R. Eddie Clutton[b], Elliot Lilley[c], Kristine E. Aa. Hansen[d] & Trond Brattelid[e]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH259RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH139RS, U.K.; [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

Translated by Shinya Ayabe[a] & Satoshi Kunita[b]

[a]Experimental Animal Division, RIKEN BioResource Research Center, Tsukuba, Ibaraki 305-0074, Japan
[b]Center for Experimental Medicine, Jichi Medical University, Shimotsuke, Tochigi 329-0498, Japan

**The PREPARE guidelines are guidelines for experimental planning and complement the reporting guidelines represented by the ARRIVE guidelines.**
**The PREPARE guidelines are broadly divided into 3 parts, each of which determines the quality of animal experiment designs.**

1. **Design of animal experiments**
2. **Consultation between animal experimenters and breeding facilities**
3. **Animal testing quality control**

**Some items may work better in a different order than the order shown below.Some items also straddle more than one of these 3 elements.**
**The PREPARE guideline checklist accommodates special experimental designs such as field trials. In addition, the PREPARE guidelines also include advice on animal care facility management, as the quality of the facility determines the quality of the experiment when conducting experiments in the animal care facility of each institution. See the full version of the PREPARE guidelines on the Norecopa website.**
**You can access information from all over the world by following links.**
**http://norecopa.no/PREPARE**
**The PREEPARE guidelines are constantly updated to the latest guidelines in response to new guidelines specific to specific animal species and experimental environments, as well as the introduction of optimal methods resulting from advances in the field of laboratory animal science.**

PREPARE checklist (norecopa.no)

| Items | Recommended contents |
|---|---|
| **(A) Design of animal experiment** | |
| **1. Literature search** | ☐ Formulate clear hypotheses and extrapolate about the primary and secondary outcomes.<br>☐ Consider using systematic reviews(Systematic literature search and analysis method).<br>☐ Decide which database to use and which informatic to consult with, and create a list of search terms.<br>☐ From the viewpoint of animal welfare, it is necessary to consider whether the animal species is appropriate for the animal used, its biological characteristics, and whether scientific questions can be clarified by experiments involving minimal pain and evaluate.<br>☐ Evaluate experimental design reproducibility and clinical validity. |
| **2. Compliance with relevant laws and regulations** | ☐ List laws, standards, guidelines, etc. in related fields such as animal experiments, animal transportation, and occupational safety and health that should be observed in conducting research and consider how to deal with them.<br>☐ Look for relevant guidance (e.g.handbook) and check its contents (e.g.EU Guidance on Project Evaluation). |
| **3. Rational considerations, balance of distress and outcomes, and humanitarian endpoints** | ☐ Prepare a general summary (experimental summary).<br>☐ Ascertain whether a record of deliberation has been made for this type of research in discussions with the Logic Committee.<br>☐ Work with based on the 3R principles (use of alternative methods, reduction in the number of animals used, and reduction of animal suffering) and the 3S (Good Science, Good Sense, and Good Sensibilities).<br>☐ Consider pre-registration of research plans and publication of negative data.<br>☐ Categorize and evaluate the degree of pain experienced by animals in the experimental design.<br>☐ Set clear humanitarian endpoints that are objective and easily measurable.<br>☐ Discuss the validity of animal death as an endpoint. |
| **4. Experimental design and statistical analysis** | ☐ Consider conducting pilot experiments, analyzing power, and setting significance levels.<br>☐ Define the experimental unit and set the number of animals to be used.<br>☐ Select randomization methods, prevent observer bias, and set data inclusion and exclusion criteria. |

Reference material

Norecopa.no HP

| Items | Recommended contents |
|---|---|
| **(B) Discussions between animal experimenters and breeding facilities** | |
| **5.Experiment purpose, experiment period, research funding, role sharing** | ☐ Meet with all relevant staff early in planning the experiment.<br>☐ Provide a rough estimate of the duration of the experiment and share information about the need for assistance with incident preparation, animal care, experimental procedures and waste disposal/decontamination.<br>☐ Present and discuss expected and potential costs.<br>☐ Develop a detailed plan of roles and cost sharing for all stages of the experimental design. |
| **6. Evaluation of animal facilities** | ☐ Conduct a field survey of the breeding facility and evaluate whether the building and equipment are sufficient.<br>☐ Hold meetings on staffing plans for unexpected situations and emergencies. |
| **7. Education and training** | ☐ Survey staff skills and competencies and assess if additional training or research is needed prior to experimentation. |
| **8. Health risk assessment, waste disposal, decontamination** | ☐ Conduct risk assessments in collaboration with animal facilities on all personnel and animals directly or indirectly affected through the experiment.<br>☐ Evaluate whether manuals and protocols are appropriate for all stages of experimental design and create new ones if necessary.<br>☐ Discuss how to store, decontaminate, and dispose of waste and filth generated during the experiment. |

Reference material

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| (C) Quality control of animal experiments | |
|---|---|
| **9. Test substances and experimental procedures** | ☐ Provide as much information as possible about the test substance.<br>☐ Consider the feasibility and adequacy of experimental procedures and the experimental skills required to perform them. |
| **10. Experimental animals** | ☐ Clarify the characteristics of the animals used for conducting animal experiments and writing articles.<br>☐ Avoid using excess animals as much as possible. |
| **11. Quarantine and microbial monitorings** | ☐ Discuss presumed health conditions, transport considerations, quarantine and isolation upon introduction of the facility, microbiological monitoring and whether the results will affect staffing plans for the animals used. |
| **12. Breeding environment and breeding managements** | ☐ Pay attention to the unique habits and necessary considerations of the animals used in cooperation with animal breeding specialists.<br>☐ Discuss acclimatization of animals used, optimal housing conditions and methods, environmental factors and whether they are subject to laboratory constraints (eg, fasting or single housing). |
| **13. Experimental Procedures** | ☐ Establish sophisticated procedures for how animals are captured, restrained and identified, and treated after the end of the experiment (returned to their original habitat or transferred to new owners).<br>☐ Acquire sophisticated experimental techniques such as test substance administration, sampling, sedation/anesthesia, and surgery. |
| **14. Euthanasia, relief from distress, reuse or transfer of laboratory Animals** | ☐ Seek advice on relevant laws, regulations, standards, guidelines, etc. early in advance of the experiment.<br>☐ Establish experimental and emergency euthanasia procedures.<br>☐ Evaluate the proficiency of staff who might perform euthanasia. |
| **15. Anatomy** | ☐ Detailed plans for each dissection step, including where to perform and how to identify animals and tissue samples. |

Reference material

Norecopa.no HP

*References*

1. Smith AJ,Clutton RE,Lilley E,Hasen KEA & Brattrlid T.PREPARE:Guidelines for Planning Animal Research and Testing. Laboratory Animals,2017,DOI:10.1177/0023677217724823
2. Kikenny C,Browne WJ,Cuthill IC et al. Improving Bioscience Research Reporting:The ARRIVE Guidelines for Reporting Animal Research. Plos Biology,2010;DOI: 10.1371/journal.pbio.1000412

English version

National Centre
for the Replacement
Refinement & Reduction
Of Animals in Reseach

# The ARRIVE Guidelines

## Animal Research: Reporting of In Vivo Experiments

Carol Kilkenny[1],William J Browne[2],Innes C Cuthill[3],Michael Emerson[4] and Altman[5]

[1]the National Centre the Replacement, Refinement and Reduction of Animals in Research, London, UK, [2]School of Veterinary Science, University of Bristol, Bristol, UK. [3]School of Biological Science, University of Bristol, Bristol, UK. [4]National Heart and Lung Institute, Imperial College London, UK. [5]Centre for Statistics in Medicine, University of Oxford, UK

The ARRIVE (Research on Animals: Reporting of In Vivo Experiments) guidelines were developed as part of the UK 3Rs Center's (NCR3Rs) work to improve the design, analysis and reporting of research involving animals. The aim is to maximize the use of published information and minimize unnecessary research. The guidelines were published in June 2010 in the online journal PLOS Biology and are now endorsed by numerous academic journals, major funding agencies, and numerous societies.

These guidelines aim to:
To improve the reporting of research using animals.
Guidance to authors on essential information to be included in manuscripts, but not absolute norms.
Be flexible to report a wide range of research areas and experimental protocols.
To promote reproducible, transparent, accurate, comprehensive, concise, logically organized, and well-organized manuscript preparation.
To enhance communication of research results to the wider scientific community.

This guideline does not aim to:

- Promote uniformity, stifle creativity, or encourage authors to adhere strictly to all items on this checklist. Some items on this checklist may not apply to all studies. In addition, some of the items in this checklist can be presented as tabular or graphical representations or flow charts (eg, the number of animals used in the experiment and the number of animals used in the evaluations and analyses).
- To serve as a guideline for research planning and conducting research. However, some items on this checklist, such as random grouping, blinding, and control dew leaves, may be considered useful in case planning. This is because the use of such items can reduce the risk of bias and increase the robustness of research.

Which fields of research do the guidelines apply to?

- The guidelines may be best suited for controlled studies comparing two or more experimental groups. In such studies, one or more groups will serve as control groups. The guidelines also apply to studies comparing the effects of different doses of a drug, or when the same animals are used as control animals (intra-individual animal studies).
- Most recommendations also apply to studies that do not include a control group.
- The guidelines are suitable for any area of biological science research using experimental animals.

How should this guideline be used?
These guidelines provide a checklist for those preparing or reviewing manuscripts intended for publication.

References;
1.Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animals Research. *PLOS Bio/*8(6):e1000412. doi:10.1371/journal.pbio.1000412
2.Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomized trials.*BMJ* 340:c332.

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This reporting guideline project was funded by the UK National Centers for Alternatives to Animal-Free Methods in Research, Relief of Animal Pain, and Reduction of Animal Numbers (NC3Rs).

Who is this guideline intended for?

- Novice to experienced author.
- Academic journal editors.
- Reviewers.
- Funding institutions.

@NC3Rs

Further information;

www.nc3rs.org.uk/ARRIVEenquiries@nc3rs.org.uk

| | Items | Recommendations |
|---|---|---|
| Title | 1 | Describe the contents of the paper as accurately and concisely as possible. |
| Summary | 2 | Accurate summary of the background, purpose of the study (including details of the species and strains used), main methods, main findings and conclusions of the study. |
| **Management** | | |
| Background | 3 | a.Include sufficient scientific background (including references related to prior research) so that the motivation and context of the study can be understood, and explain the experimental methods and rationale.<br>b.Explain why and how the animal species and models used are capable of achieving their scientific objectives and, where appropriate, the relevance of the research to humans. |
| Purpose | 4 | Clearly describe the primary and secondary objectives of the study, as well as the hypothesis to be tested. |
| **Method** | | |
| Logical statement | 5 | State the type of permit for rationale review, relevant licenses (e.g., the Animal (Scientific Experiments) Act 1986), and national or institutional guidelines for the care and use of animals involved in the research. |
| Research plan | 6 | For each experiment, briefly describe the details of the research plan, including:<br>a.Number of experimental and control groups.<br>b.Measures to minimize the impact of subjective preconceptions. This was done in assigning treatments to animals (eg, randomization of groups) and in evaluating outcomes (eg, if blinded, who was blinded and when).<br>c.Experimental unit (e.g., one animal, one group of animals, or all animals in one cage).<br>Timeline or flow chart may be useful to show how a complex study plan was implemented. |
| Experiment procedure | 7 | Accurate and detailed description of all treatments performed for the experiment and experimental groups (including controls).<br>Examples,<br>a.How (e.g., drugs prescribed and dosed, site and route of administration, anesthetics and analgesics used, including how drugs were effective; surgical procedures, euthanasia).<br>b.When (e.g. time of day).<br>c.Where (e.g. home cage, laboratory, hydropath).<br>d.None (e.g. rationale for selection of anesthetic used, route of administration, dose of drug, etc.). |
| Experimental animal | 8 | a.Details of animals used, including species, strain, sex, developmental stage (e.g. mean or median age and age range), and weight (e.g. mean or median weight and weight range).<br>b.Provide relevant information. For example, animal source, international transparency, genetic modification status (e.g., knockout or transgenic), genotype, health and immune status, not medicated or used in experiments, previous actions taken, etc. |
| Inhabitants and breeding | 9 | Provide detailed information on the following items.<br>a.Housing (type of facility: e.g. specific pathogen free (SPF): gauge or type of housing; bedding material; number of animals of the same gauge; shape and materials of fish tanks, etc.)<br>b.Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, water quality for fish, etc., type of diet, feeding/watering method, environmental enrichment).<br>c.Welfare-related assessments and interventions performed before, during, or after the experiment. |
| Sample size | 10 | a.Clearly state the total number of animals used in the experiment and the number of animals in the experimental groups.<br>b.Explain how animal numbers were determined, including details for calculating sample size.<br>c.If applicable, indicate how many times the experiment was performed. |
| Assignment of animals to experimental groups | 11 | a.Describe in detail how animals were assigned to experimental groups (including random grouping or group matching, if applicable).<br>b.Describe the order in which the animals in the different experimental groups were treated and evaluated. |
| Experimental consequences | 12 | Clearly indicate the primary and secondary experimental outcomes evaluated (e.g., cell death, molecular markers, behavioral changes). |
| Statistical method | 13 | a.Describe in detail the statistical methods used for analysis.<br>b.For statistically processed datasets, clearly state the unit of analysis (eg, one animal, one group of animals, one neuron).<br>c.Describe the methods used to assess whether the data meet the assumptions of the statistical method. |
| **Result** | | |
| Basic data | 14 | For experimental groups, report characteristics and health status of relevant animals prior to treatment or experimentation (body weight:, microbiological status, and not being medicated or used in experiments). (These information can often be tabulated.) |
| Parsed number | 15 | a.Report the number of animals in each group used for analysis. Report absolute numbers. (Example: 10/20; 50% is not acceptable.) |
| Results and evaluation | 16 | Perform and report the results of the analysis with precision (e.g. standard error or confidence interval) |
| Adverse events | 17 | a.Describe in detail any relevant adverse events.<br>b.Describe any modifications to the experimental protocol that have been made to reduce adverse events. |
| **Inquiry** | | |
| Interpretation /scientific consensus | 18 | a.Interpretation of results taking into account research objectives and hypotheses, current theory and relevant research findings (literature).<br>b.Comment on the limitations of the study, including potential sources of bias, limitations of the animal model, and inaccuracies associated with the results.<br>c.Describe the implications of the method or results for substitution of non-animal alternatives, alleviation of animal complaints, or reduction of animal numbers (3Rs) in research. |
| Generalizability/Extrapolation | 19 | Describe comments on whether and how the findings of the study can be extrapolated to other animal species or other organs or systems, including their relevance to humans. |
| Funding | 20 | List all sources of funding for the research (including grant number) and describe the role of all funders. |

ARRIVE

The ARRIVE Guidelines: Animal Research: Reporting of *In Vivo* Experiments. Originally published in *PLOS Biology*, June 2010[1]